



Title: Method for the screening of  $\alpha_2\delta$ -1 subunit binding ligands

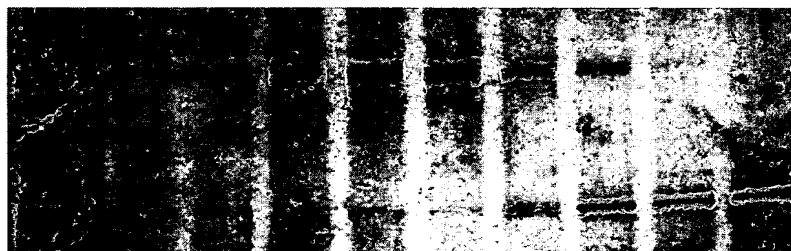
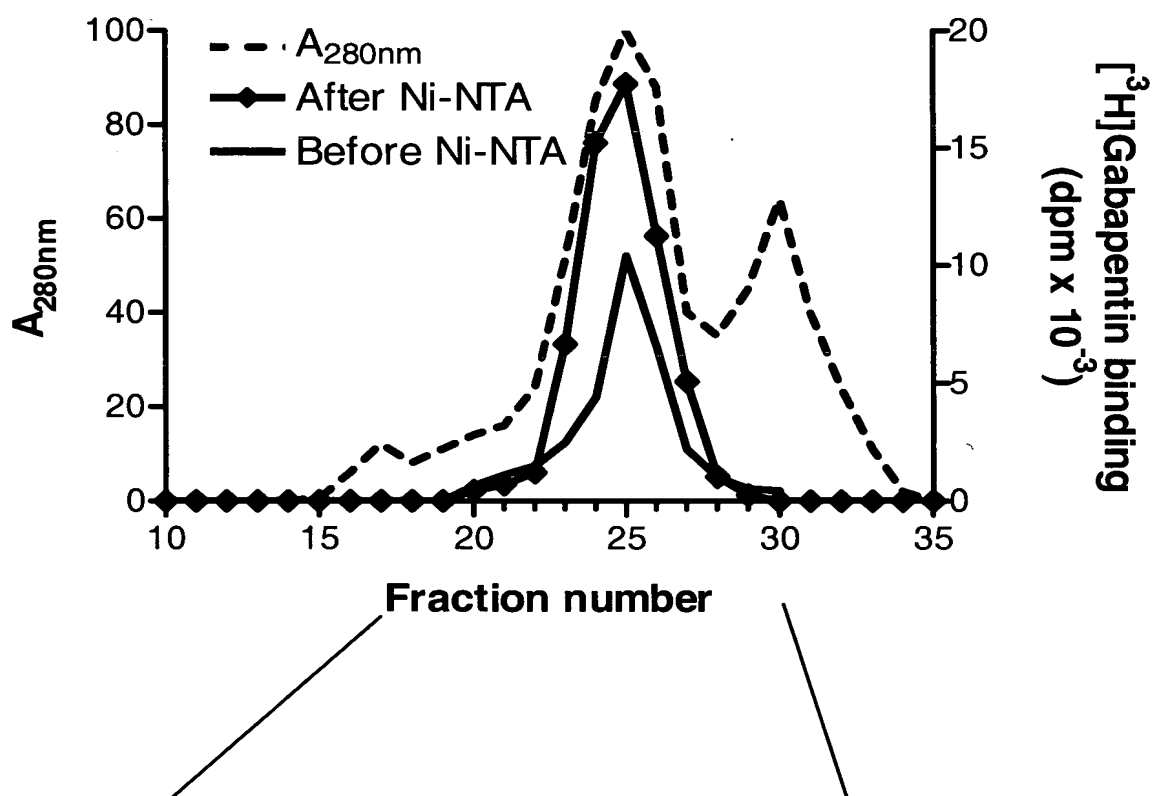
Inventor: Bertelli, Francois

Application No.: 10/090,827

Docket No.: A0000179-C1 (PC18043A)

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**FIG. 1**



~130kDa  
sol- $\alpha_2\delta$ -1b



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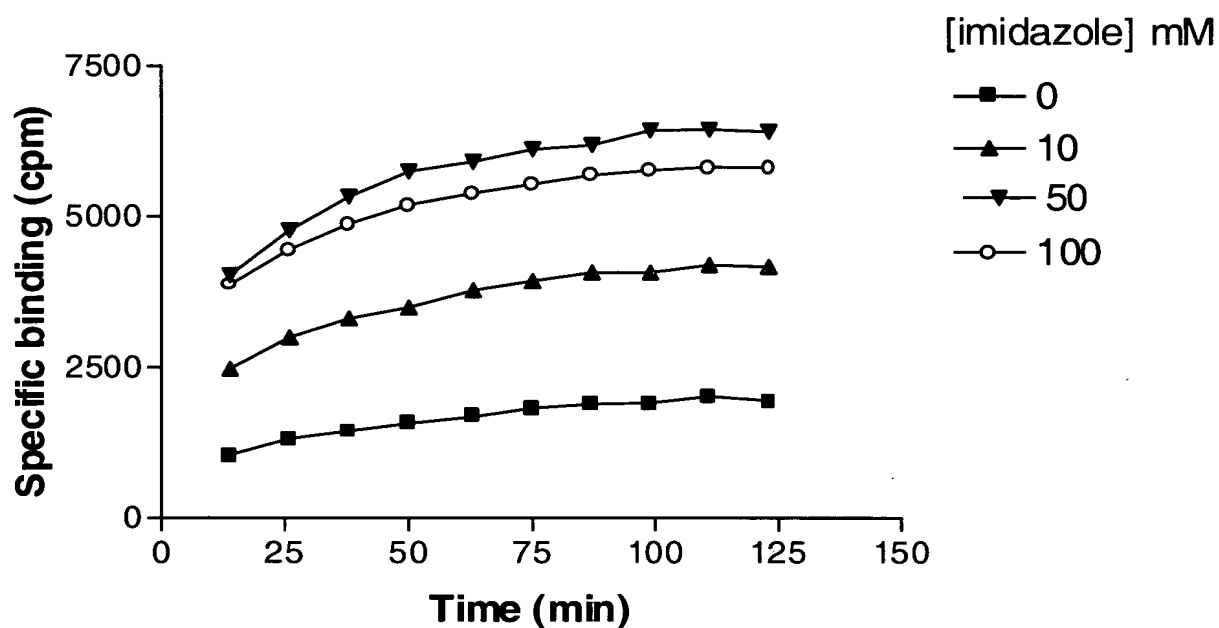
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**FIG. 2**

SPA assay of [ $^3\text{H}$ ]gabapentin (18.4nM) binding to s- $\alpha_2\delta$ -1b-6His (20 $\mu\text{l}$ ). Optimisation of Imidazole concentration in the assay.





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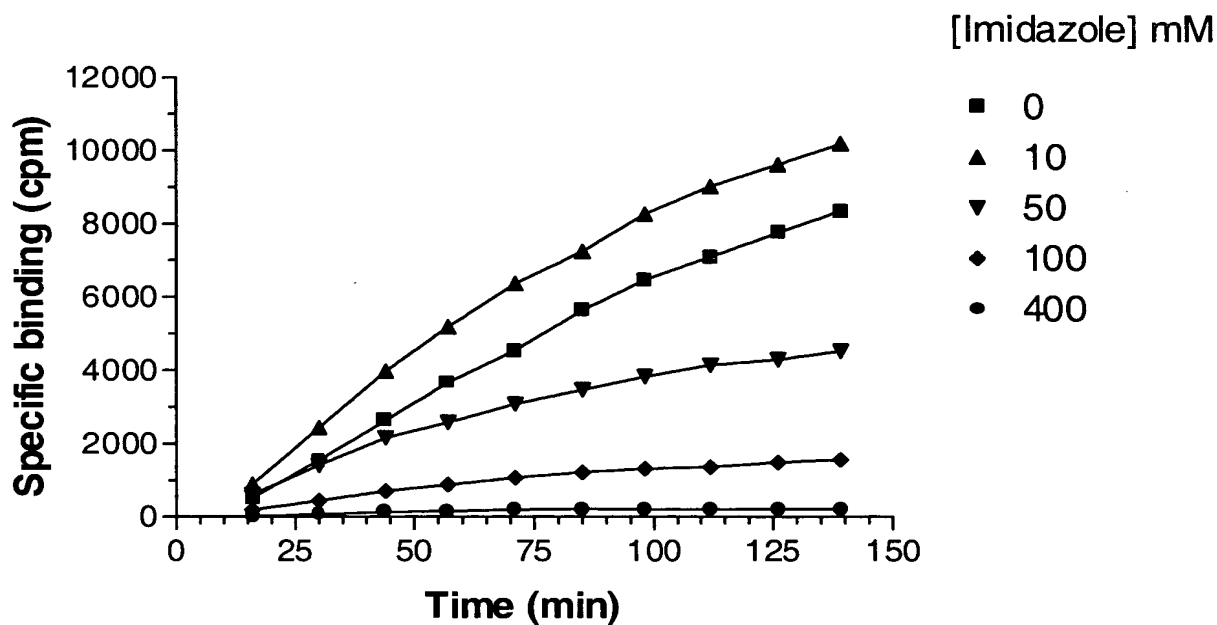
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**FIG. 3**

Flashplate assay of [ $^3\text{H}$ ]gabapentin (14nM) binding to s- $\alpha_2\delta$ -1b-6His (10 $\mu\text{l}$ ). Optimisation of Imidazole concentration in the assay.





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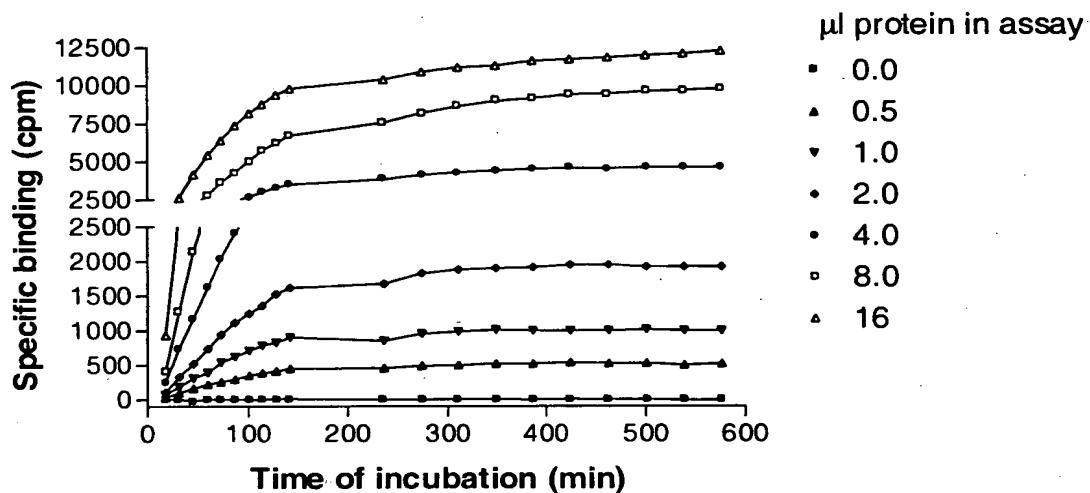
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**FIG. 4**

Flashplate time course of [ $^3\text{H}$ ]gabapentin (13nM) binding to various concentrations of s- $\alpha_2\delta$ -1b-6His. 10 mM imidazole in assay





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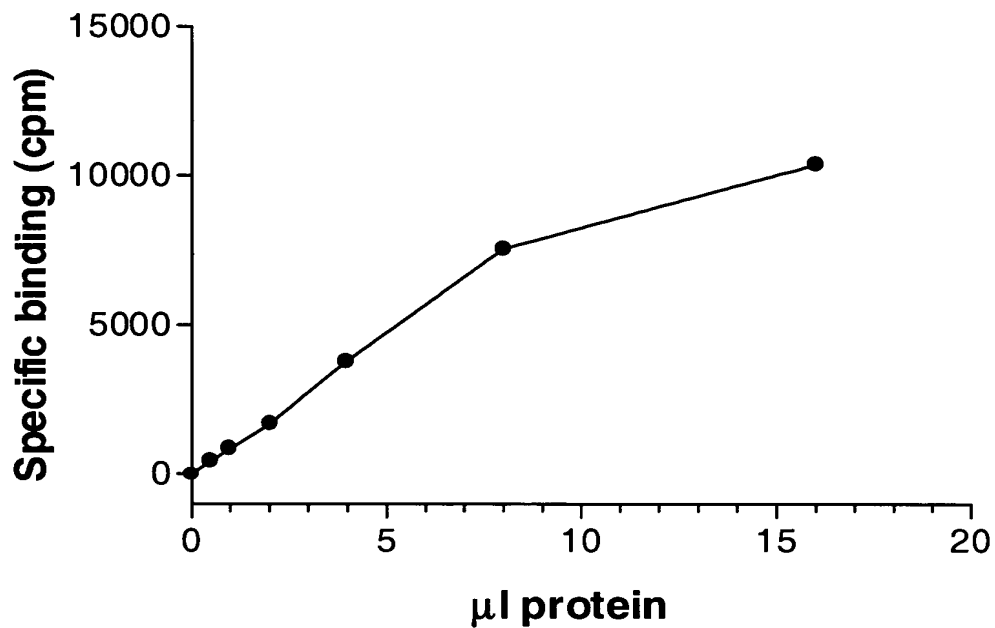
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**FIG. 5**

Determination of s- $\alpha_2\delta$ -1b-6His capacity of flashplate assay. Counted after 3hour incubation





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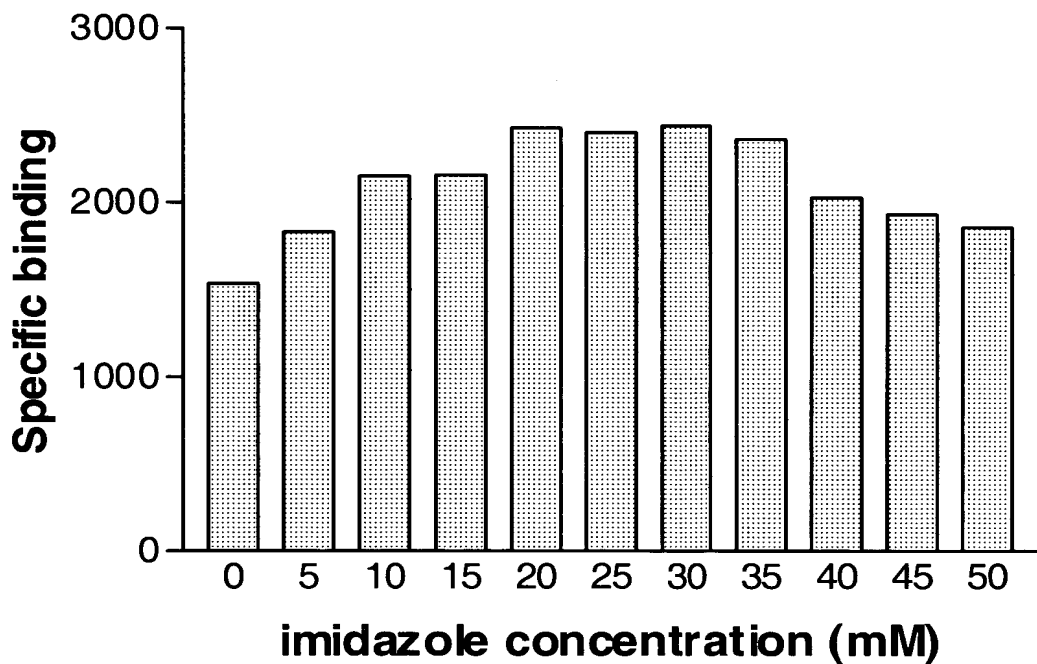
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**FIG. 6**

Determination of the optimum imidazole concentration required to maximize the [ $^3$ H]gabapentin (13nM) binding window using a constant amount of purified s- $\alpha_2\delta$ -1b-6His (2 $\mu$ l). Assayed after 3hour incubation.





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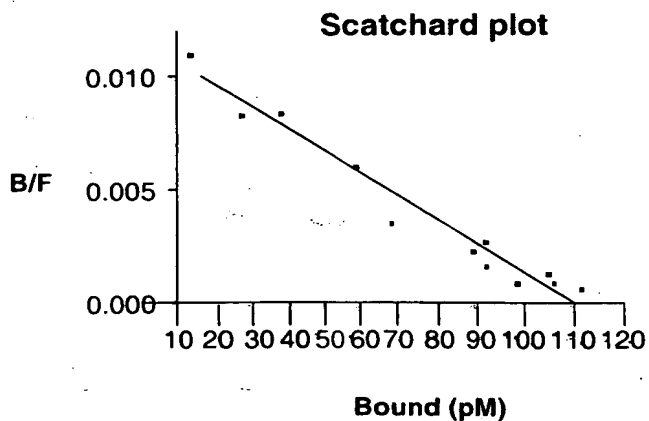
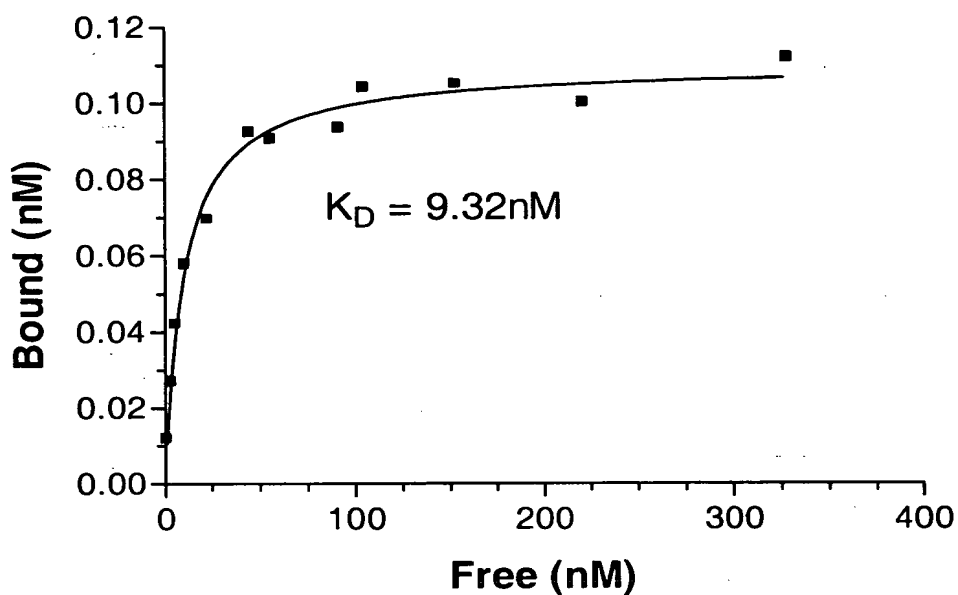
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**FIG. 7**

Flashplate assay of [ $^3\text{H}$ ]gabapentin saturation binding to purified s- $\alpha_2\delta$ -1b-6His. Assayed after three hour incubation (see table 1 for details).





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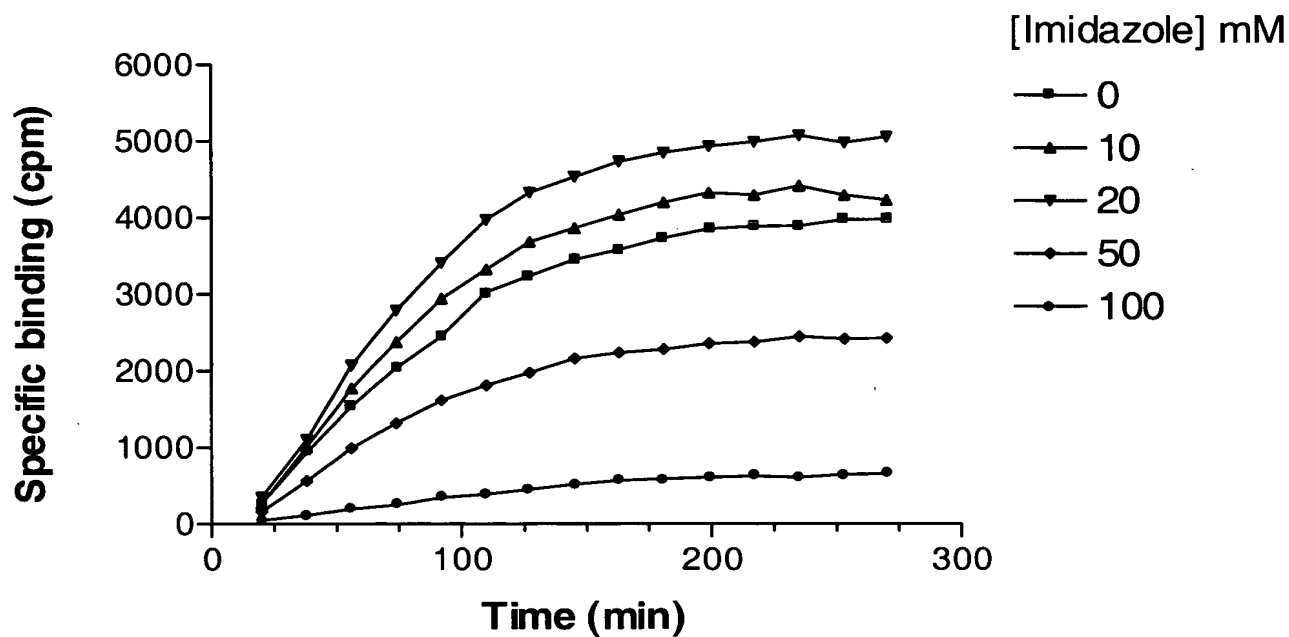
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**FIG. 8**

Flashplate time course optimisation of Imidazole concentration required to maximize the [ $^3\text{H}$ ]Leucine (10.1nM) binding window to s- $\alpha_2\delta$ -1b-6His. Assayed after three hour incubation.







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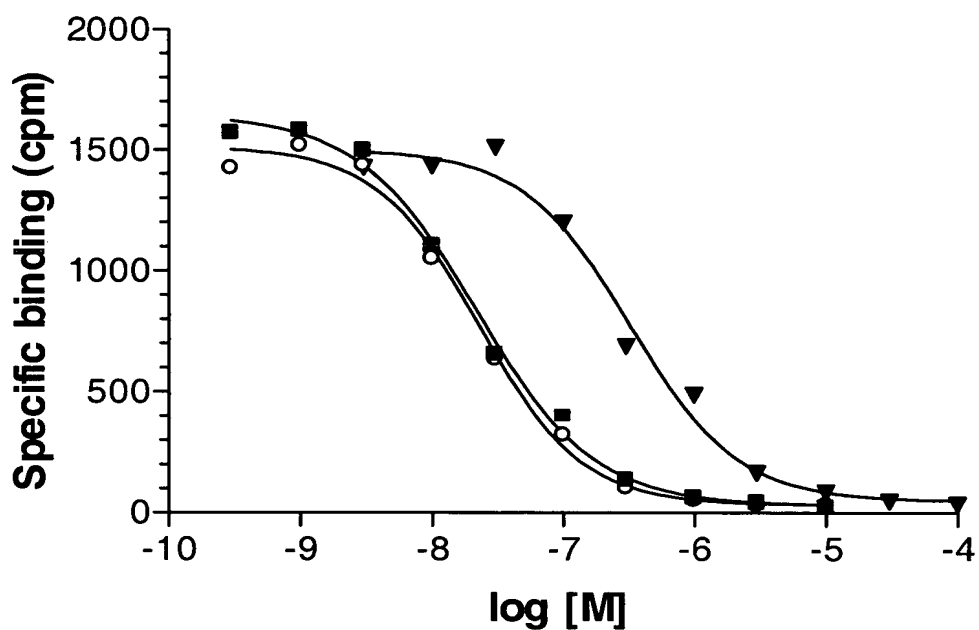
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**FIG. 9**

Competition curves of three compounds in the flashplate assay format (see table 2 for details). Assayed after 3 hour incubation.



- Gabapentin
- (S+)-3-isobutyl GABA
- ▼ (R-)-3-isobutyl GABA